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ELECTRICALLY MEDIATED TRAUMA REPAIR

ANNUAL REPORT

Dr. Richard B. Borgens

November 30, 1984

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Purdue University
West Lafayette, Indiana 47907

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and the nervous system. We have, ~~for the first time,~~ characterized the natural currents that are driven through a fracture in living bone, and ~~furthermore,~~ we have manipulated bone remodeling by purely electrical means. ~~In addition~~ (using a newly developed, completely implantable stimulating system), we endeavor to enhance or initiate nerve regeneration in the mammalian spinal cord. This work is providing a basis for the development of small DC stimulator systems that can be implanted clinically with only modest surgical endeavor. Such stimulators may initiate striking regeneration of body tissues (nerve and soft tissue) as well as greatly speed the healing of bone, cartilage, and skin.

Keywords: Regeneration (Physiology); wounds and injuries.

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Summary

In response to injury, cells and tissues drive a steady DC ionic current through themselves. This injury current and its associated electrical field is one component in the control system governing the overall tissue response to trauma. We first electrophysiologically characterize such endogenous currents and fields and, using this knowledge artificially, manipulate them. Our aim is to modulate wound healing and tissue regeneration. Our focus is trauma to the integument, hard tissue, and the nervous system. We have, for the first time, characterized the natural currents that are driven through a fracture in living bone. Furthermore, we have manipulated bone remodeling by purely electrical means. In addition (using a newly developed, completely implantable stimulating system), we endeavor to enhance or initiate nerve regeneration in the mammalian spinal cord. This work is providing a basis for the development of small DC stimulator systems that can be implanted clinically with only modest surgical endeavor. Such stimulators may initiate striking regeneration of body tissues (nerve and soft tissue) as well as greatly speed the healing of bone, cartilage, and skin.

Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, revised 1978).

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1. Statement of Problem

i) An applied, steady DC electric field is now well known to enhance the healing of both chronic fracture non-unions in humans (Brighton et al., 1979), and perhaps chronic ulcerations of the skin (Wheeler et al., 1972). These striking responses to imposed current and fields are framed against a nearly complete lack of understanding of their physiological basis. Moreover, no other applications of current to traumatized tissues have been tested for other possible clinical applications.

The problem we have attacked (supported by this contract) is twofold; first, we are pursuing measurements of natural steady voltage gradients [using a completely non-invasive vibrating electrode for measurement of extracellular current (Jaffe and Nuccitelli, 1974)] that occur in tissues in response to trauma. Using this instrument we develop an understanding of endogenous electrical responses to injury. Secondly, we are artificially applying currents and fields to traumatized tissues in laboratory mammals and analyzing tissue responses in hopes of seeing clinically significant regenerative effects. Ancillary to this we have developed an implantable DC stimulating unit that delivers current to target tissues completely free of metallic electrode product contaminations (see below).

In the original proposal, we outlined four general areas of commitment: bone and hard tissue; peripheral nervous system (PNS) tissue; integumentary lesions; and central nervous system (CNS) trauma. During the first year of the contract we have made major advances in studying the role of natural and applied currents in bone; responses of CNS neurons to applied fields; and have developed a new stimulating system for in vivo applications of current. In the continuing report, these 3 areas will bear different headings for the ease of the reviewer.

ii) Military Significance

Traumatic injury is a natural consequence of military engagement and - though less frequent - military training. Severe injury to hard and soft tissue and the nervous system present the poorest prognosis for eventual full recovery. One approach to such trauma would be to develop a means to enhance or promote rapid healing or regenerative responses in these tissues using a minimum of specialized surgical intervention. Applied DC fields are known to produce an 80% cure rate in chronic fracture non-unions (Brighton et al., 1979), however, DC fields have not been thoroughly tested to promote more rapid healing of fresh fractures. In animal studies, such fields can promote gross regeneration of hard and soft tissues (Borgens et al., 1977b, and 1979a), and striking regeneration of PNS and CNS neurons (reviewed by Borgens, 1982, see also Borgens et al., 1981) - all in lower vertebrates. None of these approaches (save the bone work) has been further tested for possible clinical application.

One could envision a small implantable DC stimulator and electrodes that could be attached to traumatized tissues with a minimum of surgical endeavor, perhaps even in field hospitals. Such may produce a striking enhancement in healing rate or nervous system regeneration. This may facilitate eventual treatment of casualties in large hospitals; may serve to shorten the time personnel (with less severe injuries) could return to active duty; and/or increase the percentage of full functional recovery in more severe injuries.

2. Background

i) Bone and Soft Tissue

The notion that hard and soft tissue regeneration may be controlled naturally (and be experimentally manipulated) by electric fields stems from studies of amphibian limb regeneration. In these studies a natural flow of wound-induced current traverses the forelimb stump after amputation and is a component in the controls of limb regrowth. The experimental support for this is as follows:

1. The current traversing the core tissues of the stump in adult frogs (nonregenerators) is strikingly reduced when compared to the density of the current (hence electric fields) within the core tissues of the stump in salamanders and newts (Borgens et al., 1977a). [This is due to a shunting of current though subdermal lymph spaces that is found in anura but not urodeles (Borgens et al., 1979c)].

2. Enhancing the fields within adult frog limb stumps (by implantation of batteries and electrodes) can initiate a measure of limb regeneration (Borgens et al., 1977b) or improve the external form of limb regeneration in hypomorphically regenerating species (reviewed by Borgens, 1982).

3. Topical applications of amiloride, benzamil, or methyl ester of lysine, chronic immersion in Na+ -depleted medium, and the imposition of a counter current within limb stumps all serve to inhibit/or retard limb regeneration, or cause it to be abnormal (Borgens et al., 1979b, Vanable et al., 1983). What all of these different techniques have in common is that they reduce the currents traversing the salamander limb stump.

4. Although there are unreconciled differences between the regeneration and development of the amphibian limb, it is probable that these processes share certain mechanisms of control. Both are characterized by the amassing of cells to form a limb rudiment (by cell division and migration), and both share certain anatomical similarities (such as the apical cap of the limb blastema and the apical ectodermal ridge of many developing limb buds). One may wonder if developing limbs and regenerating limbs share a similar phenomenology of endogenous current flow. It is significant that limb development in the larvae of both a salamander [the axolotl (Borgens et al., 1984a)] and a frog [Xenopus (Robinson, 1983)] is predicted by a local exodus of current from the exact area from which a limb will rise.

Among those tissues that responded in a marked fashion to applied electric fields (in #2 above), bone and nervous tissue were most striking.

As mentioned, clinical imposition of current using metal electrodes is known to produce healing of non-unions. In work supported by this contract, we have provided the first measurements of natural steady fracture current traversing fresh fractures made to the living bones of laboratory rodents and measured in physiological media (Borgens, 1984). These measurements were made with an ultrasensitive vibrating electrode with a sensitivity of a few tenths of a uA/cm^2 and a spatial resolution of about 30 um (Borgens, 1984, and see below). It is important to note that the natural and clinically-applied currents are similar in density, direction, and perhaps duration. This provides a possible rationale for the effectiveness of artifically-applied fields in healing non-unions; that is, that they mimic natural fracture

currents critical to fracture healing. This furthermore suggests that a common denominator in all biological non-unions may be a defect in the electrophysiology of repair.

These ideas and experiments are pertinent to this contract in that they strongly support a natural electrical control of fracture healing and furthermore suggest that applied fields may speed normal fracture healing and eventual union.

3. Research by Others

ii) Nervous Tissue

Sven Ingvar in 1920 was the first investigator to directly test the notion that an applied electric field may enhance nerve regeneration. There have been many classical experiments since this time, however, I will not review this older and historic literature on galvanotropism or galvanotaxis (see Borgens 1982). The first carefully controlled and thoughful tests of such ideas were conducted by Marsh and Beams in 1946. They observed a heightened growth response (and a guided movement) of neurites emanating from explanted and cultured chick dorsal root ganglia (DRG) toward the negative pole of an applied electrical field. Paul Weiss (1934) criticized all such experiments suggesting that applied fields orient molecules in the culture substrate and this explained the "directional responses" or neurites exposed to artifically-applied electricity. This single opinion held for many years, and until more modern times, the entire area was held to be controversial. The responses of neurons to artificially-applied fields were considered to be either artifactual or due to secondary variables independent of the imposed field.

Jaffe and Poo (1979) noted that Weiss' arguments were unsound because modern studies of electrically-induced birefrigence alterations in well-ordered macromolecules (such as collagen) demonstrate that 100's to 1000's of volts/cm were necessary to achieve realignment or reordering (such as Weiss suggested occurred in the culture substrate). Nerves in culture responded to 10-100 mV/mm. They repeated Marsh and Beam's seminal study (using chick DRG in culture) and directional responses by neurites). Moreover, they used markers in the substrate to monitor movement of the explanted ganglion (in addition to the neurites). They discovered that the ganglion mass has a proclivity to migrate toward the anode (+ pole of the applied field). If neurites facing the opposite pole (- pole) are "stuck" to the substrate at their growing tips, then they can be stretched as the ganglion moves toward the anode. This gives the impression of neurite elongation toward the antipode (cathode). When careful marking techniques are employed the results still confirmed that a ignificant (even luxurious) growth of neurites is stimulated facing the negative (-) pole of the field imposed across the culture. Other groups pursuing these studies [Sisken et al., (1981) for example] still do not focus their attention on ganglionic movement, thus most experiments by other modern groups using explanted ganglia are ambiguous and I will not review this less rigorous literature.

Recently, three more culture experiments provide unequivocal proof of electrically-induced growth responses n nervous tissue. Robinson and McCaig (1981), Hinkle et al. (1981), and Patel and Poo (1982) have observed the responses of individual differentiating neuroblast neurites in culture to applied fields. Individual cells are obtained from disaggregated Xenopus neurula stage embryos and such cells develop into neurons in culture. Individual growing

neurites will bend through great arcs to deviate their axis of growth toward the negative pole of the applied field. The rate and amount of neurite growth was enhanced in this vector, and always parallel with the long axis of the applied field. In the latter study, if the axis of the field was changed during the experiment, neurites (originally) growing toward the cathode, reversed their direction of growth and recriented themselves - growing towards the new position of the negative pole. These experiments were well-controlled, fields were applied with salt bridges, and cells were grown on tissue culture plastic (no substrate was used). Taken together with the in vivo studies these studies constituted formal proof that a DC applied electric field can grossly influence nerve growth and promoted our continuing studies using the lamprey spinal cord system. Lamprey larvae possess large individually identifiable neurons in their spinal cord. The brain and spinal cord can be completely removed to a simple organ culture where it prospers for about 1 week (the CNS of the lamprey does not have a blood supply intrinsic to the cord or brain, but is nourished by diffusion from vessels at the surface. Thus it does very well in a fortified organ culture environment). The lamprey regenerates its CNS neurons, and functionally recovers from a complete cord transection in about 200 days. We first decided to investigate if lamprey cords (or individual CNS neurons) naturally produce large electric currents in response to injury. Using the vibrating electrode we measured enormous currents of injury and extracellular fields about the lesion in transected lamprey spinal cord (Borgens et al., 1980). Such currents last indefinitely and were found to enter the cord parenchyma and the open bores of severed neurons. We next decided to impose a large steady field across complete cord transections to see if we could modulate or enhance spinal cord regeneration.

Using long flexible saline bridges (or wick electrodes), we imposed an electric field (or the order of 10 mV/mm) across the completely severed spinal cord of lamprey larvae for 5 to 6 days, with the anode rostral and the cathode caudal to the lesion (Borgens et al., 1981). Sham-treated animals were treated identically to experimentals except that no current was delivered to the tissues. In our preliminary experiments, of the 15 current-treated and 15 sham-treated animals, 11 and 13, respectively, survived to provide data. At about 55 days post-transection, we assayed the responses to these electrical applications by a combination of simultaneous extracellular and intracellular recording of action potential (AP) propagation across the lesion. After electrical records were taken (by antidromic and orthodromic stimulation and recording across the lesions), the axons responsible for the intracellular records were injected with the fluorescent dye Lucifer yellow. (The recording intracellular microelectrode was filled with this dye.) This allowed us to compare the anatomy of those descending reticulospinal neurons that propagated AP's across the lesion with those neurons that did not. I will summarize our main findings: (a) In 73% of the animals treated with electric current, APs eleicited by extracellular stimulation of the whole spinal cord were propagated in both directions across the lesion. (b) In most (69%) of the sham-treated controls, APs did not propagate across the lesion in either direction. This is not surprising since only modest axonal regeneration is usually observed at 200 days post-transection in the lamprey. This gave us a reasonably unambiguous baseline with which to compare the effects of our treatments. (c) Intracellular recording was combined with extracellular recording and fluorescent dye labeling of individual cells to characterize the giant axons responsible for propagating AP's across the lesion. Axons which conducted spikes antidromically across the lesion site were found to traverse it; in a few cases, terminate within the lesion. Few axons terminating within, and no axon ending proximal to the lesion could be fired by stimulating

distal to the lesion and recording just behind the brain. These tests demonstrate that the increased occurrence of AP's propagating across the lesion in electrically-treated cords, can be ascribed to the increased number of axons regenerating into or beyond the lesions area. (d) The greatest number of fluorescently-labeled axons in current-treated cords were found within or through the lesion by about 55 days post-transection. Most of a comparable population of axons in the sham-treated controls had ended proximal to the lesion not entering it at all. (e) The morphology of most of the terminal ends of identifiable giant axons found in experimentally-treated spinal cords was indicative of actively growing regenerating fibers. Most of the ends of control fibers were relatively undifferentiated morphologically, appearing as axons that are in a less active g wth state, perhaps even in stasis. Altogether, we felt that these results encouraged further testing of the hypothesis that applied electrical fields may enhance the regeneration of CNS nerves in vertebrates. I hope to have persuaded the reviewer that applied electrical fields can induce significant growth responses in damaged or developing vertebrate axons (by whatever mechanism), both <u>in vivo</u> and <u>in vitro</u>. In fact, a close comparison of these experiments to similar such experiments testing the effects of nerve growth factor (NGF) on axonal outgrowth and directionality should convince the critical reader that certain responses (such as directional guidance) are quite profound when applied fields are used as an effector. [I wish to stress that until our recent studies, partially supported by this contract, (see below), applied DC fields have not been rigorously tested at all in the mammalian spinal cord. We wish to continue these studies and determine if applied fields may significantly alter the character of CNS regeneration in the mammalian spinal cord.]

4. <u>Approach to the Problem</u>

Our general approach to the electrical control of regeneration has been this: we first <u>characterize</u> natural currents of injury using a vibrating probe system in regenerating and non-regenerating tissues. This provides physiological insights into the overall electrical responses to injury and an electrophysiological understanding of the system. We then try and manipulate such endogenous currents and in doing so try and manipulate tissue responses to injury (reviewed by Borgens 1982). If we manipulate endogenous currents and fields with implanted stimulating units we <u>always</u> use a current delivery system that will not contaminate target tissues with electrode products.

<u>Stimulator Design</u>

Implantable stimulator assemblies are fabricated in the following manner. The voltage source is a lithium manganese dioxide 3 volt unit (Sanyo CR1220). This unit provides 30 milliamp hours capacity and is very small in size (2 mm stack height, 12.5 mm diameter, and 0.8 gm). The battery is connected in series to the rest of the following components by means of silver conductive epoxy: a small fixed resistor, a 3 terminal adjustable constant current source (National Semiconductor, LM-334), and at each pole, a 3-4 mm length of chlorodized silver wire (AgAgCl contacts to the salt bridge). Two millimeters of the AgAgCl contacts are masked off, and the unit is dipped in a bakelite electronics potting compound (stycast 261, Emmerson and Cummings). (This provides structural durability to all electrical connections.) A 10 cm long, 1 mm O.D., 0.06 2 I.D. silastic tube is filled with a mammalian Ringers - agar slurry and a cotton string. Two of these are coiled tightly (but not compressing the tube) and each is attached at one end to the AgAgCl battery connection. The tube is slipped over the AgAgCl wire and glued in place with medical grade elastomer. This connection is sound and the voltage source <u>and</u> the coiled salt

bridges are dipped into medical grade elastomer and stored in zirconium chloride in Ringers. Prior to surgical implantation a long (ca 12-14 cm) wick electrode (0.025 I.D. silastic tube filled with Ringers and a cotton string) is slipped into the open bore of the salt bridge and the connection electrically and structurally sealed with medical grade elastomer. The unit is now ready to be implanted into the peritoneal cavity of laboratory animals, the long electrodes can be trimmed to size after they are surgically routed beneath the skin to the exposed spinal cord or bone, for example.

The National Semiconductor LM-334 constant current source sets total current output of the stimulator at that magnitude determined by the fixed resistor in series with the salt bridge and wick electrodes. Current output is held steady even in the face of changing resistances at the electrode bores (due to clots, encapsulation, etc.) or alteration in the wick's total resistance due to sizing it to the animal. This, plus the nonpolarizing AgAgCl contacts, provide an exceptionally dependable and long-lived implantable system.

We deliver current to the tissues by wick electrodes in series with large bore salt bridges to completely eliminate the possibility of tissue contamination by electrode products (produced by electrolysis at the Ringers - metal interface). The rate of movement of (principally) metallic components will depend on the total voltage drop across the salt bridge. Therefore, we use a large bore, low resistance (in ohms) bridge between the stimulating electrodes and the AgAgCl contact to the voltage source.

The rationale is that we wish to know unambiguously that whatever responses we observe are in fact due to current and fields and not due to electrode produce contaminants. Thus we deliver current to target tissues via "aqueous wires" where charge is carried by electrolytes (as in body fluids). Another advantage: Since electrode product contaminants (principally at the anode) are cytotoxic, this system will not poison or destroy delicate tissue.

The Ultrasensitive Vibrating Probe

The vibrating probe is an extracellular electrode which measures voltage gradients outside individual living cells or whole animal tissues. This is done by vibrating a small metal sphere (10-30 um diameter) between two points typically 10-30 um apart while measuring the voltage at the two extremes of vibration. This is accomplished by tuning the frequency of vibration to the frequency of amplification using a phase-frequency lock-in amplifier. The current density component in the medium at the center of vibration and along the axis of vibration is directly proportional to this measured voltage gradient

$$[I(\mu A/cm^2) = \Delta V(v)/\Delta r(cm)\rho(\Omega cm)]$$

Thus, the current density or net ion flux at any specific region can be directly measured with minimal cellular disturbance by vibrating the probe just outside any membrane and perpendicular to it at that region, and multiplying by an appropriate surface extrapolation factor which adjusts for the field fall-off between the membrane surface and the center of probe vibration. Simply stated, ions entering or leaving the membranes must pass through the external medium of constant resistivity. Current passing through a resistance generates a voltage (albeit quite small in most cellular cases) which the probe measures. This small voltage is proportional to the net ion flux passing along the axis of probe vibration.

This technique has been described in detail (Jaffe and Nuccitelli, 1974) and has been successfully applied to a wide variety of cell types and whole animal studies (for example, see Borgens, 1984).

We also possess another version of this instrument that we call the "macroprobe." The metallic electrode is vibrated between positions typically 300 um apart at about 40 Hz. Its resolution is less than the ultrasensitive probe discussed above, but is more rugged. It can be held by hand and gas-sterilized for use in the surgical ward.

Using this approach (understanding endogenous injury currents then manipulating the system) has proved effective in past studies in lower vertebrates. For example, an understanding of naturally-produced injury currents and electrical fields in salamander limb regeneration (Borgens et al., 1977a; Borgens et al., 1984b) and in neural regeneration (Borgens et al., 1980) has allowed us to effectively stimulate nerve regeneration in the lamprey CNS (Borgens et al., 1981) and initiate the regeneration of limbs on adult frogs (Borgens et al., 1977b; Borgens et al., 1979a).

During the first year of this contract we have 1) characterized the endogenous fields in mammalian fractures; 2) developed a new DC stimulator (see description above); and 3) have imposed fields across intact mammalian bone (in adult rats) and across lesi- al mammalian spinal cords (in adult guinea pigs).

5. Results

Endogenous Fracture Currents

We have made the first measurements of a steady DC current traversing intact and damaged long bones in mice. These results have been published as an article in Science, and I direct the interested reader to this publication (Borgens, 1984). Here I will only summarize our main findings:

1) Intact living bone drives a substantial electric current through itself. Current enters the primarily cartilagenous end regions of bone, and enters and leaves the shaft.

2) A fracture to a bone produces an immediate and large leak of current into the lesion. These densities (on the order of 100 uA/cm^2) decay to a stable level of about 5 uA/cm^2, which may persist indefinitely.

3) The large and declining currents are independent of cellular metabolism and are produced by a deformation of bone substance, while the steady plateau of about 5 uA/cm^2 is driven by a cellular battery.

4) This steady flow of charge through a lesion is largely carried by chloride ions, with a less substantial contribution made by Na+, both being actively taken into the bone compartment from the extracellular fluid.

5) Though current always enters the fracture gap, the overall geometry of current flow around and through a lesioned bone is highly variable. This observation casts grave doubt on the widely-taught concept of predictable potential gradients existing along the surfaces of a bone after a fracture.

6) These studies support the hypothesis that load-induced voltages in physiological bone may be produced by streaming potentials, and also suggest that there is a "bone-membrane," capable of pumping ions and maintaining the ionic milieu of bone different from body fluids.

7) Finally, endogenous fracture currents are of the same polarity and of roughly the same magnitude as clinically-applied currents which are successful in treating chronic fracture non-unions. This furthermore suggests that the defect in biological non-unions may ultimately be a defect in the electrophysiology of repair.

Field Effects on Intact Bone

Since all clinical and basic studies of the effects of DC fields on bone have employed wire (metallic) electrodes, we decided to test if chemically pure current and its associated electrical field could alter bone remodeling. We implanted DC stimulating units into large adult rats, routed wick electrodes to the leg, secured them to the intact femur, imposed a field of about 1-10 mV/mm across the femur, and observed responses of the bone at 3 weeks post-implantation.

Our analysis of this experiment is still continuing, however, our preliminary observations are these:

1) The imposed field indeed strikingly effects bone deposition. We have observed (histologically) an exaggerated deposition of bone at the cathode (negative wick electrode) when compared to sham-treated animals (controls).

2) We have also noted a small protuberance of bone on the endosteal surface (within the marrow cavity) of the diaphysis beneath where the electrode is secured externally. This suggests current penetrates bone and can affect even the endosteal surface.

Field Imposition on Transected Dorsal Column Lesions in Mammalian Spinal Cord

We have imposed fields of about 10 mV/mm across completely transected dorsal column tracts in adult guinea pig spinal cord (mid thoracic transection). Animals are sacrificed at 50 to 60 days post-transection and analyzed for regeneration of these ascending long tract neurons into or across the plane of transection. The control group is sham-treaed guinea pigs. In order to unambiguously know the exact plane of the original transection, we use a "Foerster device" implanted into the lesion at the time of transection and removed after perfusion fixation (see Foerster, 1982). After removal, small (ca. 50 um diameter) holes are left in the cord so that after horizontal longitudinal sectoins are made, one can determine the exact plane of transection. To analyze the cords morphologically we have developed a technique where we fill all of he dorsal column neurons with the intracellular marker horseradish peroxidase (HRP). We section large, rectangular blocks of spinal cord (20 mm long x 100 um thick) containing the lesion, process the cord for visualization of the HRP reaction, and clear the cord. What we have at the end of this process is large whole mounts of spinal cord, semi-transparent, in which only the dorsal column neurons are visualized in their entirety. Since we fill dorsal columns with the marker about 1 to 2 cm caudal to the lesion, we only view long tract axons arising from this level. It is important to note that no dorsal column neurons arising from higher thoracic levels or neurons of a local origin in and around the lesion are

visualized using this technique. Therefore, filled neurons seen within the lesions or traversing it would have to have occurred by axonal regeneration.

Our preliminary experiments suggest that indeed, dorsal column neurons can be visualized within the lesions and in 3 cases we have unambiguous evidence of fibers crossing the lesion. We are also becoming aware that the character of the lesions the collagenous and astroglial scar may be different in current-treated animals. We are presently pursuing these studies in two directions. We hope to optimize the neuronal response to applied fields by imposing larger fields across transected dorsal columns; secondly, we are now analyzing the morphology of the scar on current-treated and sham-spinal cord treated animals.

6. Conclusions

Natural and Artificial Currents in Fracture Repair

It seems probable that these currents are relevant to bone repair, remodeling, and perhaps growth, and not just an epiphenomenon. Support for this statement lies in the fact that weak artificially-applied currents can heal non-unions, and otherwise modulate bone resorption and deposition. All of the work using artificial current applications to bone have employed metal electrodes, which will contaminate the local tissues with metallic ions and thus it cannot be stated unequivocally that current (by itself) mediates these processes. The critical experiment that was sorely needed is to duplicate such effects in living bone by using wick electrodes or salt bridges to carry the current to the bone, eliminating metal ion contaminants as possible effectors.

The experiment in preparation (cited above) in which we have grossly affected bone remodeling using wick electrodes to deliver current to the tissue will (when published) be instrumental in demonstrating that current flow (in biological systems current is the movement of electrolytes) can indeed affect bone deposition.

If endogenous currents are in fact controls in bone repair, it is interesting to ponder this possible redundant control system. The substantial incurrents produced by deformation of bone matrix fade to a stable level in an organ culture dish, however in the animal, load will always be exerted on the lesion, and this component of the injury current may be a more chronic feature of the electrophysiology of injury. For bones that may receive little loading (rib cage bones) or deformation during movement, a stable cellular battery will still pull current into the injury (however, probably of a lesser magnitude).

Two obvious, and interesting, questions arise from the view that steady ionic current may be a control in vertebrate fracture healing: Do biological non-unions arise because of a lack of such current?; and do the clinically-applied currents mimic the naturally-produced currents in character? The latter question we can answer with some authority: Yes, the two are roughly similar in density, and polarity. Negative electrodes are necessary within the fracture gap of a non-union to achieve healing, thus extracellular current will be pulled into a gap. The naturally-produced fracture current enters the lesion as well. Total current on the order of 10 to 20 uA per electrode is used clinically. Since multiple electrodes are used, and the geometry of the various lesions are highly variable, it is difficult to do anything but a rough calculation of the density of current produced within the fracture gap. It is reasonable to assume the unit area of ununited fractures in human long bones may be greater than a square centimeter. A 4 electrode (10 uA per

electrode) configuration would produce a density of 40 uA/cm^2 within a lesion of 1 cm^2. Thus, it is reasonable to speculate that the current densities produced by electrode insertion will be on the order of 10's of uAs per cm^{-1} - a value well within the range of current densities naurally produced by fractures. We next plan to test the effectiveness of current in speeding the healing rate of "fresh" fractures.

CNS Regeneration in Response to Applied Fields

We have evidenced (and are, at present, building a firm experimental basis) that an artificially-applied field can induce regeneration of central (spinal cord) neurons. Moreover, we have preliminary evidence that the character of the scar (produced by the original transection) is different in current-treated animals when compared to sham-treated animals.

If neuronal regeneration can be induced in the spinal cord and brain, and we are able to optimize this effect (to be able to initiate a more luxurious growth of neurons) then there resides the promise that some degree of functional recovery may accompany neuronal regeneration through the scar. We chose dorsal column neurons for our preliminary tests because of their precise anatomy and for other experimental reasons. Eventually, we hope to move these studies into cortico-spinal neurons (motor columns) incorporating behavioral tests as well.

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GLOSSARY OF TERMS

a) Dorsal Columns. These large spinal cord tracts are bundles of neurons which project into the spinal cord from segmental ganglia lying just outside the cord itself. Sensory information (largely) is carried to the brain by these tracts which ascend the cord.

b) Laminectomy. Surgical exposure of the spinal cord within the vertebral column.

c) Neurite. A general and nonspecific term for a neuronal process.

d) Wick electrode. An aqueous "wire." Stimulating electrodes fashioned from a silastic tube, filled with mammalian Ringers and a cotton string (the "wick"). Thus, current is carried to the tissues by a conductive solution similar to body fluids and not by metallic wires (which contaminate the tissues with electrolysis products). The wick insures that there is electrical continuity in case air bubbles form and partially occlude the inner diameter of the tube.

e) Orthodromic and Antidromic stimulation and recording.

Experimentally-evoked action potentials whose conduction pathway is in the same direction as natural conduction are orthodromically stimulated. For example: orthodromic stimulation of a motor neuron would involve stimulating near the soma (or ganglion) and recording at the periphery. Antidromic stimulation and recording would be the reverse of this regimen.

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